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Quantification of optically granular texture of benthic foraminiferal walls

RITSUO NOMURA

Foraminiferal Laboratory, Faculty of Education, Shimane University, Matsue, 690-8504, Japan (*e-mail: nomura@edu.shimane-u.ac.jp*)

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Abstract. Three main textures may occur in optically granular walls of hyaline calcareous foraminifera: mosaic granular, jagged granular, and minute granular. The size and shape of the optical granules within them indicates that these wall textures are intimately related to the crystalline arrangement of the units and their elements, and also to the wall thicknesses of the foraminiferal tests. Highly complex minute-granular textures are observed if the foraminiferal tests are large and walls are thick. In general, crystallographically compound and intermediate wall structures correspond to the minute-granular texture. Both formsize (ratio of perimeter to area) and Shannon-Wiener index for polarized crystal units explain these different wall textures well. This study suggests a method for quantification of wall textures based on image processing.

Key words: benthic foraminifera, crystal unit, ecology, optical textures, test walls

Introduction

Hyaline calcareous walls of benthic foraminiferal tests consist of small crystallites and their assembled crystal units. Hansen (1968, 1970) clarified these crystalline structures by scanning electron microscope studies, and in the 1970s several authors examined these foraminiferal test structures in diverse foraminiferal taxa (e.g., Banner and Williams, 1973; Stapleton, 1973; Bellemo, 1974a, b; Conger *et al.*, 1977). Features of the crystalline structures in test walls are revealed by high interference colors under polarizing microscopy. Wood (1949) introduced the terms radial and granular structures for these optical features of foraminiferal walls. Nomura (1983, 1988) further recognized variations in each optical texture, and subdivided granular structure into mosaic, jagged, and minute (Figure 1.1-1.3), and radial textures into distinct and indistinct. These subdivisions of the granular walls are based on the optical grain size and the structure. Although a clear-cut distinction between them is sometimes difficult, the mosaic granular has larger and less jagged appearances than the jagged one. Optical grains of the minute granular are conspicuously small and complicated in comparison with the mosaic and jagged ones. These optical textures clearly reflect the complexity of crystalline structures consisting of various optical axes of the crystal units and their elements (Nomura, 1983).

The optical textures of foraminiferal walls have mainly been utilized for systematic purposes. Loeblich and Tappan (1964, 1974, 1987) used optical features of foraminiferal walls for their hierarchical classification. This classification now needs to be reexamined in view of increased knowledge. Apart from its application to foraminiferal systematics, wall texture can be used to assist in interpretation of foraminiferal ecology and paleoecology (Nomura, 1988, 1997). In a preliminary report (Nomura, 1997), I suggested that granular textures show variations corresponding to the preferred ecology of individual species. The best example is found in the ecological difference between epifaunal and infaunal species. Mosaic granular texture is mainly found in infaunal taxa, and minute granular texture is seen in epifaunal taxa (Nomura, 1997). It is empirically understood that the crystal units of foraminiferal tests show variations in their perimeter and area in polarized light. As there are gradual changes among the mosaic, jagged, and minute granular textures, however, application of these optical textures to ecology and paleoecology is not definitive. Information on the ecological and ontogenetic characters of the wall textures is still limited.

In order to clarify the optical grains of these textures by quantitative analyses, I examined live and dead specimens having different textures and different growth stages. Observations of the wall texture using a polarizing microscope are particularly useful on account of the simple methodology employed. Definition of analytical methods is needed however to perform reliable comparisons of foraminiferal wall textures.

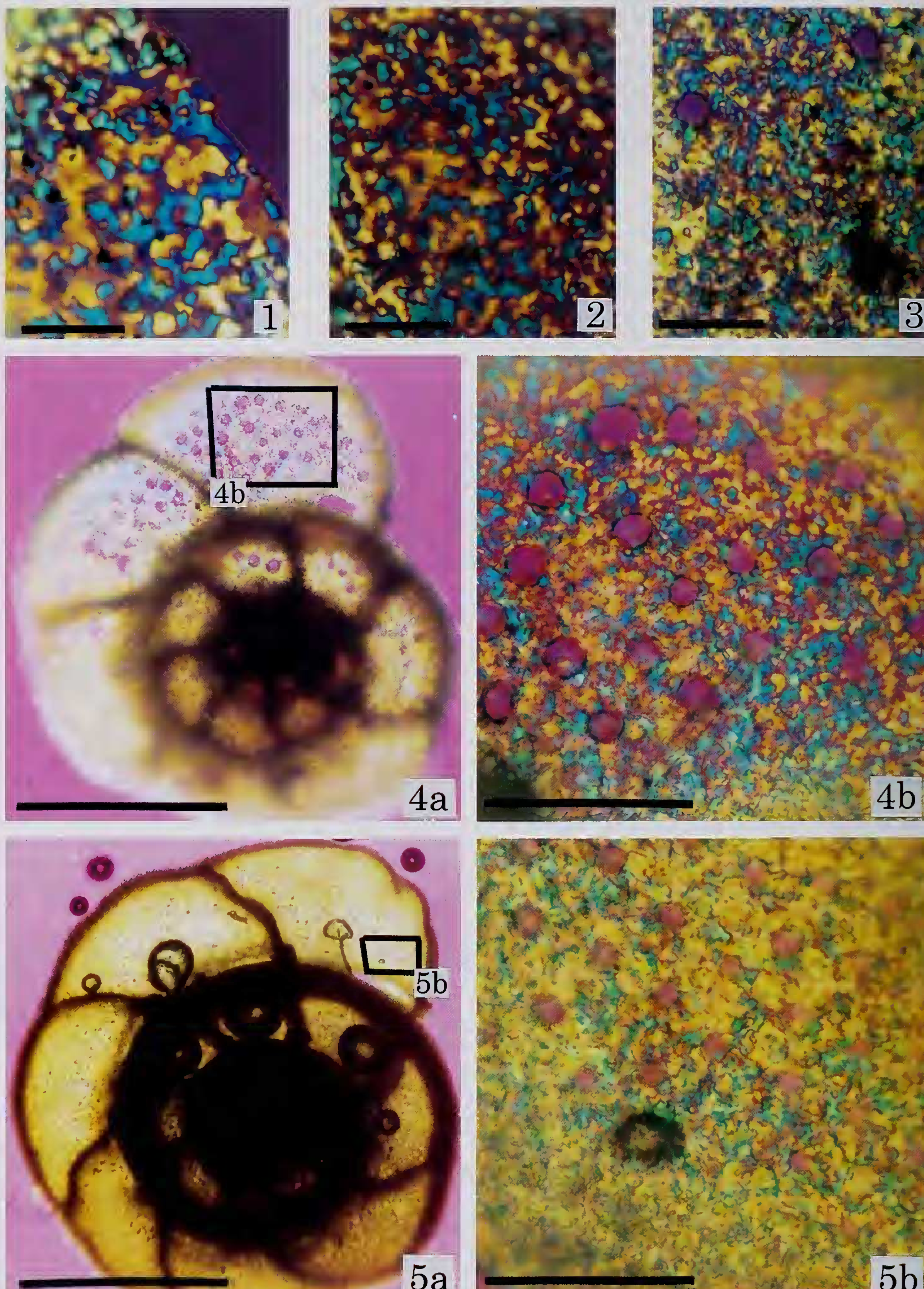

1
2
3
4a
4b
4b
5a
5b
5b

Table 1. Species examined in this study.

taxa	test size (mm)	wall thickness (μm)	depth in m (sample)	condition
Anomalinoides glabratus (Cushman)	0.24-0.56	2.5-15.0	54(HK-1*)	dead
Cassidulina reniforme (Norvang)	0.14-0.20	1.0-2.5	194(HN3-9*)	dead
Chilostomella oolina Schwager	0.38-0.49	1.0-3.8	Pliocene Iioka Formation, Choshi **	fossil
Cibicides lobatulus (Walker and Jacob)	0.38-0.78	6.8-18.2	54(HK-1*)	dead
Cibicides refulgens Montfort	0.22-0.71	4.5-12.5	54(HK-1*)	dead
Cibicidoides pseudoungerianus (Cushman)	0.56-0.83	8.0-11.3	99(HK-4*)	dead
Cibicidoides wuellerstorfi (Schwager)	0.23-0.72	1.5-20.0	99(HK-4*)	dead
Elphidium advenum (Cushman)	0.20-0.62	2.0-12.5	54(HK-1*)	live, dead
Fursenkoina pauciloculata (Brady)	0.29-0.83	1.3-6.2	54(HK-1*)	live, dead
Globocassidulina oriangulata Belford	0.17-0.33	2.0-5.0	99(HK-4*)	dead
Gyroidina orbicularis d'Orbigny	0.20-0.49	2.5-14.0	99(HK-4*)	dead
Gyroidinoides nipponicus (Ishizaki)	0.22-0.37	3.8-4.0	54(HK-1*)	dead
Heterolepa subhaidingeri (Parr)	0.40-0.86	6.3-19.5	99(HK-4*)	live
Nonionellina labradorica (Dawson)	0.22-0.39	1.0-4.7	150(CB4-1*)	live
Nonion manpukuziensis Otuka	0.27-0.66	2.5-7.5	54(HK-1*)	dead
Oridorsalis umbonatus (Reuss)	0.18-0.46	1.0-4.3	150(CB4-1*)	dead
Pullenia bulloides d'Orbigny	0.16-0.32	2.8-5.0	150(CB4-1*)	dead
Paracassidulina neocarinata (Thalmann)	0.206-0.32	2.2-5.5	99(HK-4*)	dead

* KT-90-15, Tansei-maru Cruise, off Shimane and Yamaguchi Prefectures, Sea of Japan (Ocean Research Institute, Univ. of Tokyo)
** Well preserved

Methods

The last chambers of live and well preserved dead specimens of 18 foraminiferal species were analyzed (Table 1). Foraminiferal tests were first embedded in glycerin jelly and covered with a thin glass cover slip as in standard preparation for microscope observation. Tests were crushed and fragments of the final chamber walls were arranged carefully by pressing the glass under a binocular microscope while the jelly was liquid enough to allow the wall pieces to move.

Observations and measurements of the crystal units were carried out under a polarizing microscope at a magnification of ×400. Measurements of the wall thickness were made on final wall fragments set vertically on the glass at the magnification of ×1000, after wall texture photography. Crystal units were observed most effectively using the first-order interference colors arising from insertion of a gypsum plate. The image analysis was carried out using Winroof (version 3.5.2; Mitani Corporation, 2000), which runs on Windows computers. The observations were made at an angle of 45° to the optically positive or negative orientations of the crystal units. Two methods were used to quantify the texture image. Firstly the perimeters and areas of manually selected crystal units were measured to calculate the ratio of perimeter to area (A/P ratio or formsize) (Nomura, 1997). Ten to twenty crystal units were measured for each wall piece. Because this method is subjective, sometimes selection errors can be made, especially when the unit is not clearly differentiated from neighboring units. To avoid such selection errors, I applied a second method that detects crystal units after color processing which disintegrates the original color image into RGB (red, green, and blue).

Crystal units are more effectively distinguished in the G (green) image at specific threshold values (Figure 2). Thresholding is a brightness discrimination, which selects pixels belonging to features of interest (Russ, 1990). Possible values range from 0 to 255. A block model of the green image, in which the peaks correspond to the brightness intensities, is shown in Figure 2.2. Selection of the crystal unit areas is thus critically controlled by the threshold values. Various threshold values were examined to find the best texture images. Statistically, pixel brightness has a characteristic frequency distribution for each texture, and usually shows a normal distribution (Figure 2.4). The following formula was used to determine the threshold value for each specimen examined: Threshold value = Average threshold value + Standard deviation. Between 100 and 500 areas of selected crystal units were counted for each specimen.

The selected unit images were subsequently converted to binary images (Figure 2.3) and their areas, perimeters, and formsizes then calculated. These measurements were

← **Figure 1.** Variations of optically granular wall texture. **1.** Mosaic-granular texture of *Chilostomella ovoidea*. Scale bar = 50 μm. **2.** Jagged-granular texture of *Elphidium advenum*. Scale bar = 50 μm. **3.** Minute-granular texture of *Cibicidoides pseudoungerianus*. Scale bar = 50 μm. **4a, 4b.** Horizontal section of a small *Heterolepa subhaidingeri* and close-up of the final chamber wall showing the minute-granular texture. Scale bar: 4a, 200 μm, 4b, 100 μm. **5a, b.** Horizontal section of a larger *H. subhaidingeri* and close-up of the final chamber wall showing indistinct crystal unit boundaries. Scale bar: 5a, 500 μm, 5b, 100 μm.

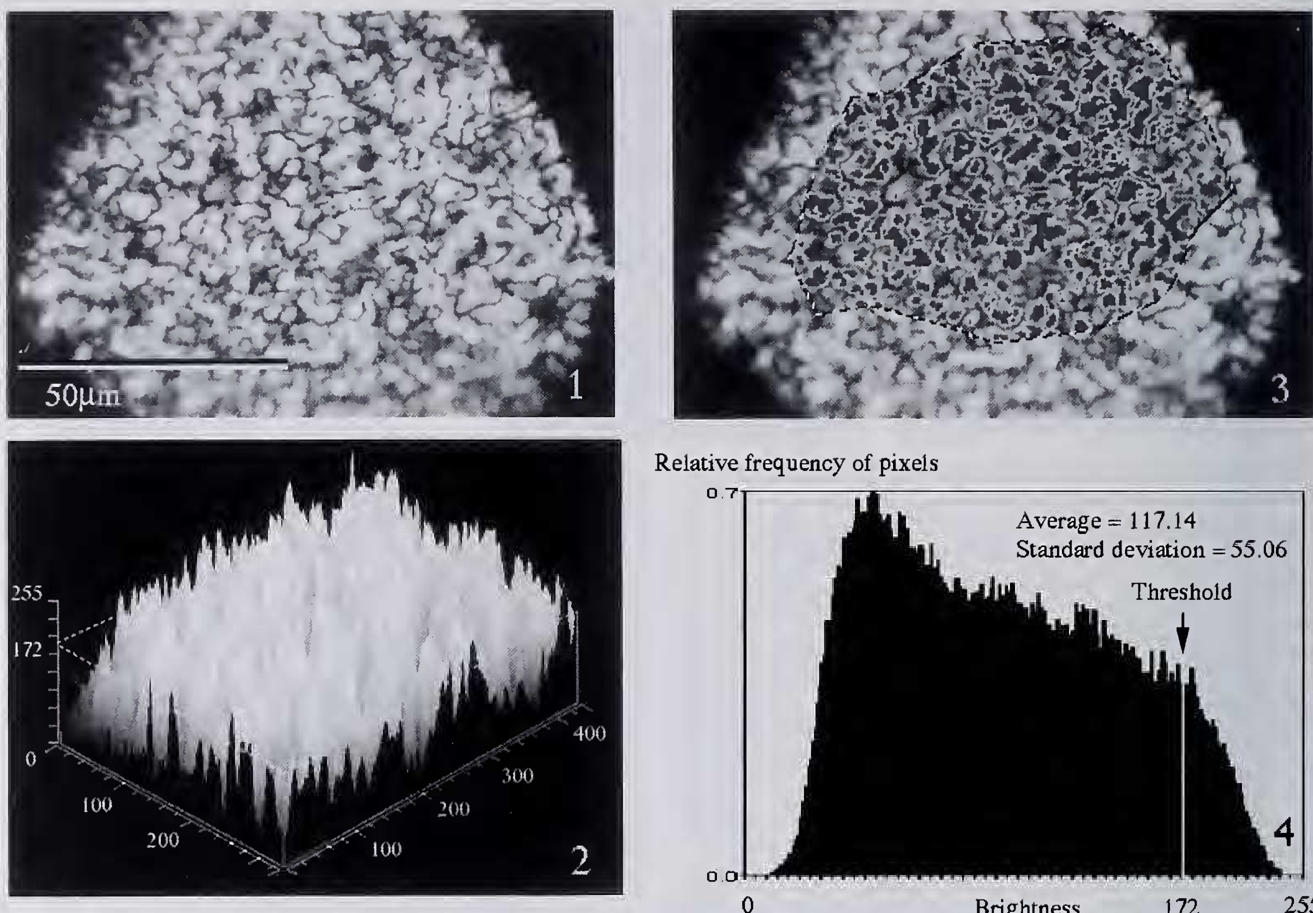


Figure 2. Explanations for the image processing of *Fursenkoina pauciloculata*. **1.** Green image of the texture separated from the blue and red images. **2.** Block diagram of the green image. Thresholding is the brightness value used to distinguish particular images from others, ranging from 0 to 255. Dotted horizontal lines indicate the threshold value (172) in this analysis. **3.** Binary image of the crystal unit areas at threshold value 172. **4.** Histogram showing the relative frequency of pixel brightness (0–255). The averaged brightness is 117.14 and the standard deviation is 55.06. Thresholding at 172 (sum of the average and the standard deviation) accounts for 20.35% of the selected texture.

based on the binary images at threshold values of 130–240. The formsize of each crystal unit is calculated by the formula: Formsize = 2·(Area)/(Perimeter). Values are $\geqq 1$. If the formsize is 1, the crystal unit is perfectly circular and its radius is 1. The A/P ratio (Nomura, 1997) is a simple expression of this formsize.

The areas of the selected crystal units show a wide variation between 1 to 2000s pixels. Statistical values with high standard deviation make the comparison of the formsize unreliable. However, both the number of the selected areas and the number of pixels they contain represent the difference between the textures, so that they conform to the concept of ecological heterogeneity that accounts for the amount of order or disorder in any given part of the wall. The Shannon-Wiener information function (H') is herein applied to evaluate the diversity of the textures:

$$H' = -\sum_{i=1}^{N}(Pi)(\log_2 Pi)$$

where N is the total number of the crystal units selected and Pi is the proportion in the i th-selected area to the total areas selected. Higher values of H' indicate the textures are characterized by a more complex crystalline arrangement, whereas lower values represent textures consisting of more simple arrangements.

It is difficult to measure the thicknesses of fixed parts of the walls, because breakage occurs randomly during crushing. Analysis was limited to flat pieces of final chamber walls. Sutural areas consist of complicated crystalline structures showing interwoven crystal units and elements. Such areas are not suitable for this analysis. Wall thickness is proportional to test size, and so increases in individuals with growth, even though it varies between foraminiferal species. Thus, careful selection of wall fragments is necessary if reliable results are to be obtained.

Results

The smaller specimens examined here (maximum diameter 0.15–0.25 mm) usually have final chamber walls between 1.0–5.0 μm thick. However, mature specimens of species such as *Chilostomella ovoidea*, *Nonionellina labradorica*, and *Cassidulina reniforme* may also have thin walls (< 5.0 μm). Large specimens (0.4–0.9 mm diameter) of species such as *Heterolepa subhaidingeri* show a wide range of wall thickness (6.3–19.5 μm; Table 1). Wall thickness differs between taxa, and appears to be reflected in wall texture. Thin-walled specimens show well defined boundaries between crystal units displaying distinct blue, red, and yellow areas, but thicker specimens have indistinct boundaries, and blue and red areas are much reduced. These color changes are caused by the interference order of polarizing light, because internal refraction of incident light occurs in every crystal element in the unit and at the unit boundary. Thin-walled crystal units present first-order interference color, but thicker walls containing assembled crystal units produce multiple interference. Brightness of pale yellow images thus increases with increasing wall thickness.

Change of optical texture in relation to the wall thickness can also be seen within individuals as they grow. As observed in *Cibicidoides pseudoungerianus* tests of differing

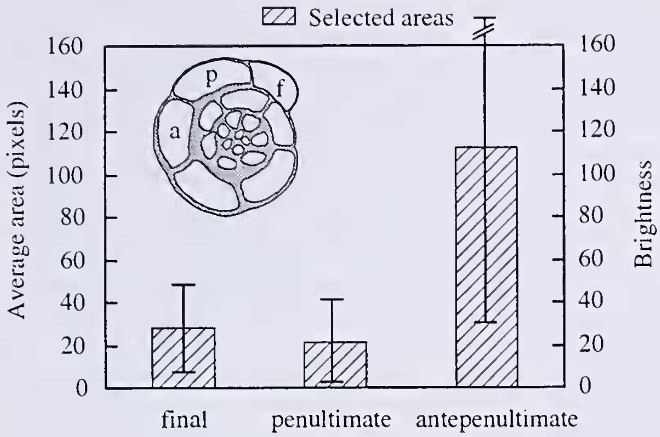


Figure 3. Results of averaged areas with standard deviations for the last three chambers of a sectioned *Oridorsalis umbonatus*. Threshold value is 130. Increased areas in the antepenultimate chamber are caused by indistinct boundaries between the crystal units. f: final chamber. p: penultimate. a: antepenultimate.

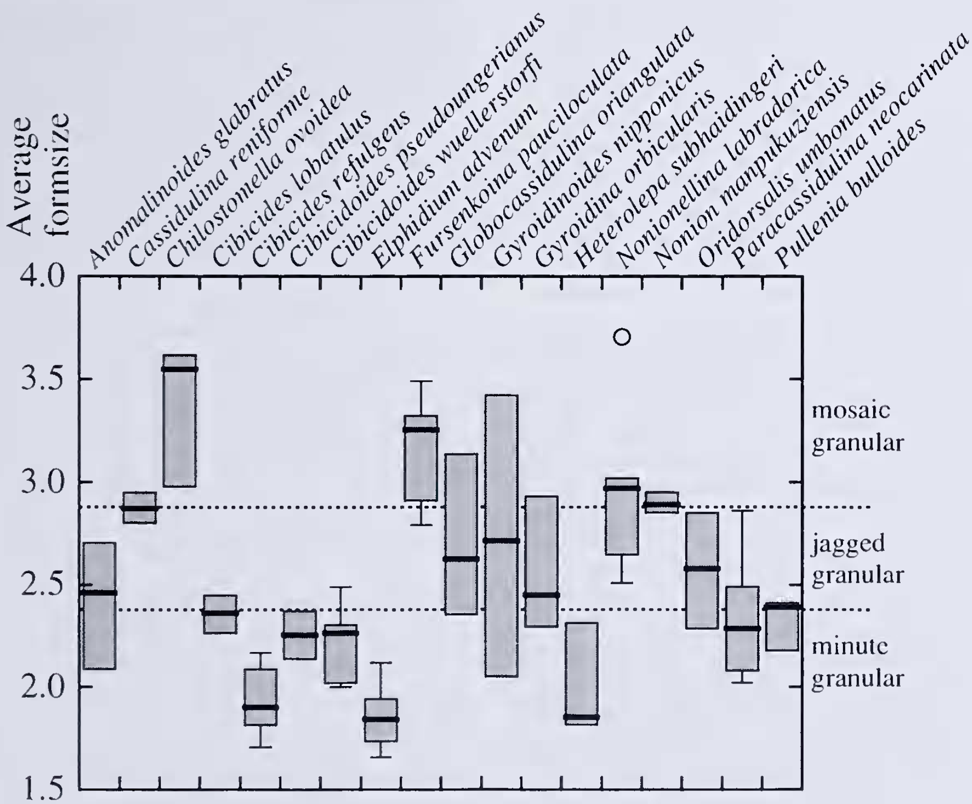


Figure 4. Average formsize in each species. Many species have a wide variation in formsize, which is related to the different textures. Mosaic-granular texture is typified by formsize of > 2.8; formsize of minute-granular textures are < 2.4; and values for jagged-granular texture lie between 2.4 and 2.8. Hatched boxes enclose 50% of the formsizes and the tops and bottoms of the box mark ±25%. Thick horizontal line indicates the median. Small circle is an exceptional value.

size (Figure 1.4, 1.5), unit boundaries of crystal units in the final chamber wall can be easily distinguished. In thicker walls, higher interference makes the boundaries less clear (Figure 1.5b). As noted above, the yellowish color in the antepenultimate chamber is caused by higher-order interference colors. The variations in average areas of the selected crystal units and the brightness in the walls of the last three chambers of *Oridorsalis umbonatus* are shown in Figure 3. The average area of the crystal units in the antepenultimate chamber walls is four to five times larger than that in the walls of the final and the penultimate chambers. Areal increase in the antepenultimate chamber walls is clearly related to the brightness, which makes the unit boundary indistinct. Clear discrimination of the crystal units is possible in the final chamber, where wall thickness is usually < 3–4 μm.

Based on the formsize, mosaic-granular texture occurs in *Chilostomella ovoidea* and many immature specimens (i.e., small specimens) of *Cassidulina reniforme*, *Fursenkoina pauciloculata*, *Globocassidulina oriangulata* , *Gyroidinoides nipponicus*, *Gyroidina orbicularis*, *Nonion manpukuziensis* and *Nonionellina labradorica*. This texture is recognized by formsizes of over 2.8 (Figure 4). The walls of *Nonionellina labradorica* show atypical mosaic-granular texture, where either the optically positive or negative conditions are dominant in the apertural face. Optical axes of the crystal elements are equally arranged over large areas, but are oblique to the test surface. This texture can also be seen in taxa having larger apertural faces, such as *Nonion* and *Nonionella*. Typical minute-granular texture is shown by most species of the genera *Cibicidoides*, *Cibicides*, and *Heterolepa*. This texture reflects the original complexity of their crystalline arrangement. In the Cibicidinae (Bellemo, 1974b, 1976), this is termed compound and the intermediate structure. Similar formsize is also seen in other mature specimens of *Anomalinoides glabratus*, *E. advenum*, *G. oriangulata*, *G. nipponicus*, *G. orbicularis*, *O. umbonatus*, *Paracassidulina neocarinata* and *P. bulloides*, except for *Chilostomella ovoidea* and *Cassidulina reniforme*. However, their crystalline structures differ slightly from those of the Cibicidinae in having larger crystal units and herringbone structure (e.g., Nomura, 1983). Thus, the minute-granular texture is formed by the original complex crystalline structure and by an apparent feature of thick walls consisting of mosaic and jagged-granular textures. The boundary between minute-granular and jagged-granular may be around a formsize of 2.4 (Figure 4). Jagged-granular texture is usually recognized between 2.4 and 2.8.

These three wall textures show wide variations in the measured formsize values. In particular, thinner walls (< 5 mm) are characterized by high standard deviation values (Figure 5). Gradual changes between the different textures also occur. Excepting the Cibicidinae, most species show three differing textures according to the growth stages of the individual: mosaic-granular texture corresponds to the stage of new chamber formation or the younger growth stage of individual foraminifera; jagged- and minute-granular textures correspond to the full-grown stages of individuals.

A significant relationship is indicated between modified formsize (formsize divided by the square root of the number of selected crystal units) and wall thickness (Figure 6.1). This relationship can be expressed as an exponential, with r = 0.68. The formsize is divided by the square root of the number of crystal units because formsize is dependent on the number of selected areas. Mosaic granular textures are characterized by low numbers of selected crystal units and higher formsizes, whereas minute-granular textures have larger numbers of crystal units and lower formsize values. The results clearly indicate that larger formsizes have thinner walls, whereas specimens with smaller formsize values have thicker walls and/or originally smaller and complex crystal units. Shannon-Wiener information theory is the other quantitative expression to account for the heterogeneity of selected units that consist of large and small areas. The result of this information function is opposite to the relationship between formsize and wall thickness (Figure 6.2). It is thus negatively correlated with modified formsize at a statistically significant level ($p < 0.001$) (Figure 7). If the Shannon-Wiener information index is higher, then the modified formsize is smaller, and textures are complex. Conversely, if the information index is lower, then modified formsize is higher and textures are simpler.

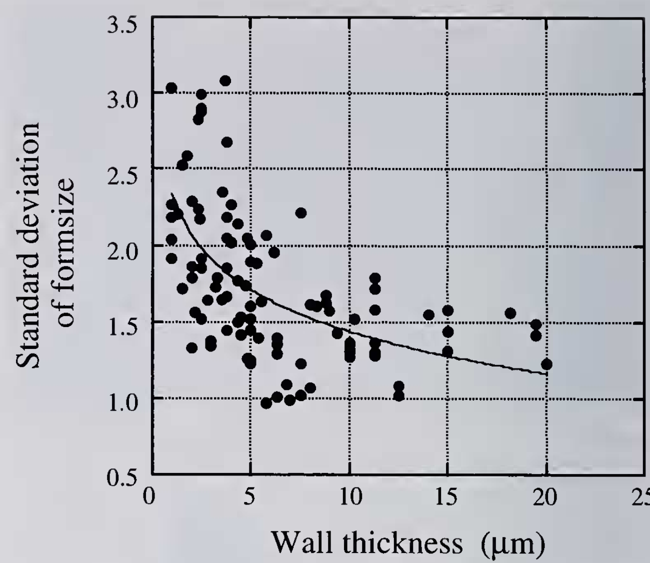


Figure 5. Plots of standard deviation of formsize and wall thickness showing large variations in thinner test walls.

As a result, distribution of respective wall textures on the formsize overlaps among different species, due to changes in the wall texture through growth. Little change in the wall texture is observed between the final and the preceding walls, as well as among different-sized specimens of thinly walled species such as *Chilostomella ovoidea* and *Cassidulina reniforme*. These species are characterized by having larger original crystal units and additional thin laminae in walls formed subsequently.

Discussion and Conclusions

Mosaic, jagged and minute granular optical wall textures result from the arrangement of crystal units and the ele-

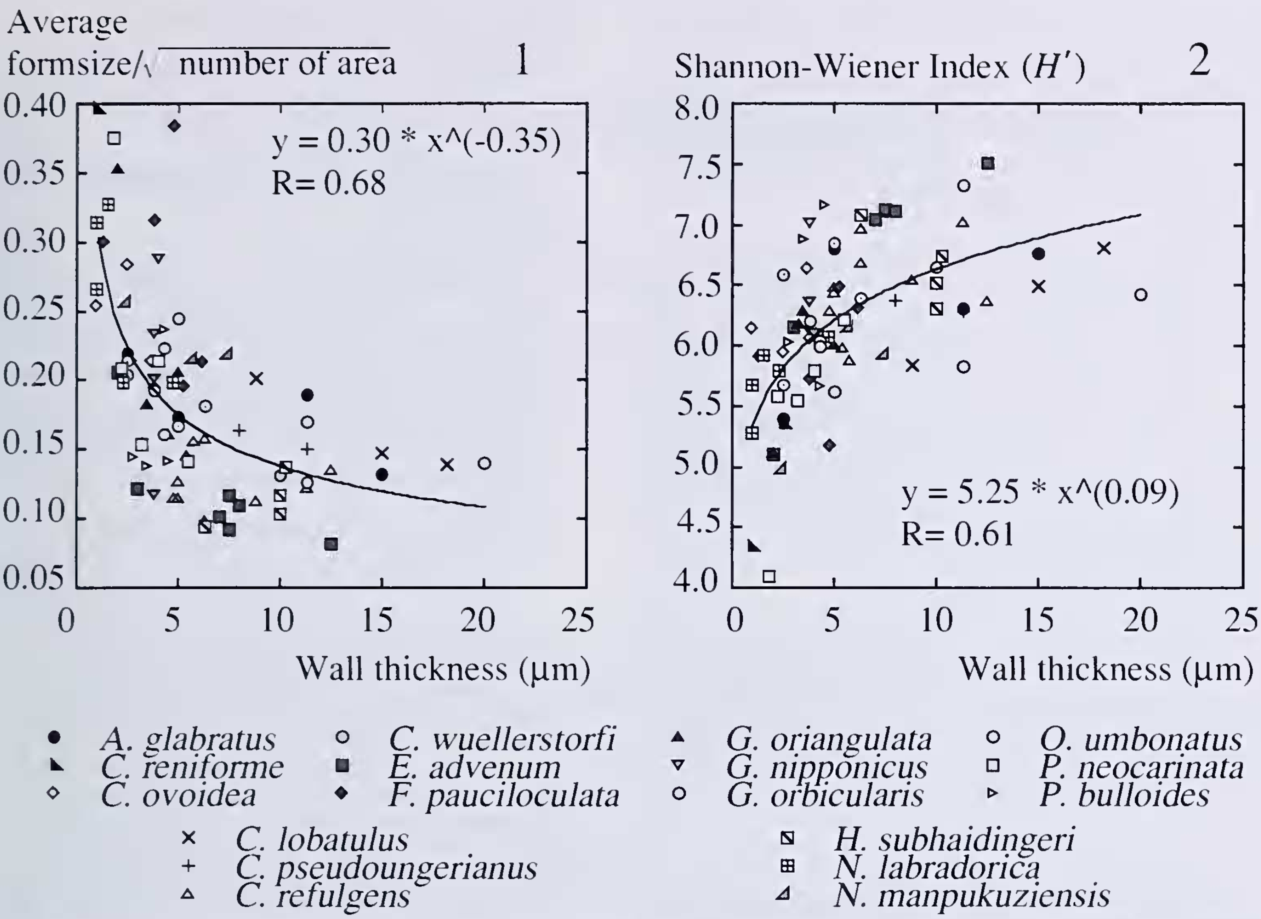
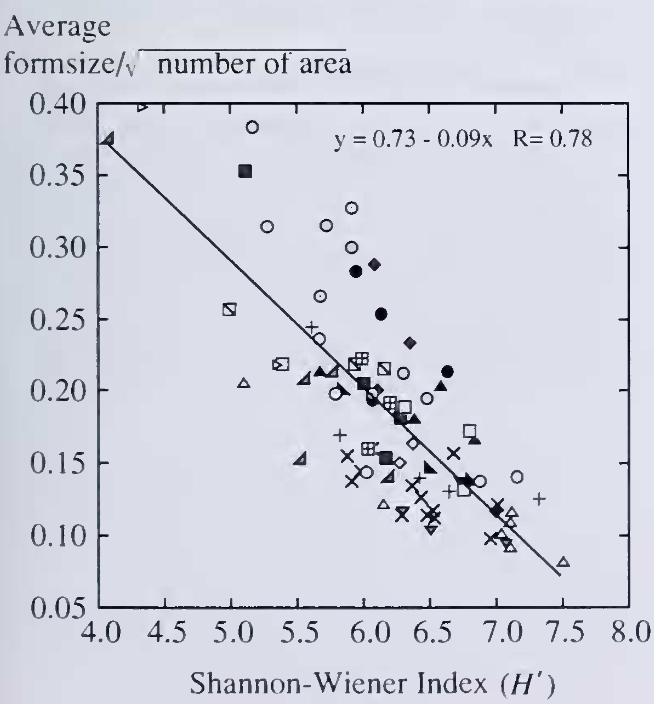


Figure 6. **1.** Plots of average formsize and wall thickness of the species, showing a negative exponential relationship. Formsize is here divided by the square root of the number of the area. **2.** Plots of the Shannon-Wiener index (H') and the wall thickness of the species examined show a positive exponential relationship.



ments within any wall thickness. Mosaic-granular texture is formed by larger crystal units and thinner walls, and was once named "clumpy crystalline structure" (Nomura, 1983). Jagged-granular texture is correlated with "intricate crystalline structure" (Nomura, 1983). Minute-granular texture is formed by two types of crystalline structures: 1) intricate crystalline structures within thicker walls, and 2) complex arrangement of crystal elements such as the compound and intermediate structures of Bellemo (1974b, 1976). Ratios of the perimeter to the area of the selected crystal unit have been introduced as a method of quantitatively discriminating these wall textures (Nomura, 1997). However, initially this method used manual selection of crystal units and thereby sometimes produced errors. Criteria for the selection of crystal units are needed. The present study confirms that the intimate relationships between optical texture and crys-

← **Figure 7.** Plot of average formsize and Shannon-Wiener index (H') showing a negative relationship with a statistically significant correlation coefficient (r=0.78). Formsize is here divided by the square root of the number of the area. Symbols as in Figure 5.

talline structure can be recognized in walls showing first-order interference colors. Even in this case, image processing is required to overcome individual variations. Several adjoining crystal units may apparently form large single units in polarizing light. Such units must be eliminated to make realistic measurements and comparisons. The thresholding proposed is a simple method of discriminating various texture images.

Classification of optically granular texture in hyaline calcareous foraminifera (Nomura, 1988, 1997) is not only a species character, but is also related to the wall thickness of the foraminiferal test and the complexity of crystalline structures. In general, thinner walls show mosaic-granular texture, whereas thicker walls and complex crystalline structures (compound and intermediate) exhibit minute-granular texture. Jagged-granular texture is present in walls of intermediate and moderate thickness. To evaluate these optical textures, the relationships between formsizes of the crystal units were examined for differing growth stages of foraminiferal individuals of selected species. The results suggest that the formsize of the crystal units shows a gradual change in accordance with the crystalline complexity of the test walls in different foraminiferal growth stages. The relationship between formsize and the wall thickness is statistically significant and exponential. Shannon-Wiener information theory is applicable for quantification of the textures, and the Shannon-Wiener index is negatively correlated with the modified formsize parameter.

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